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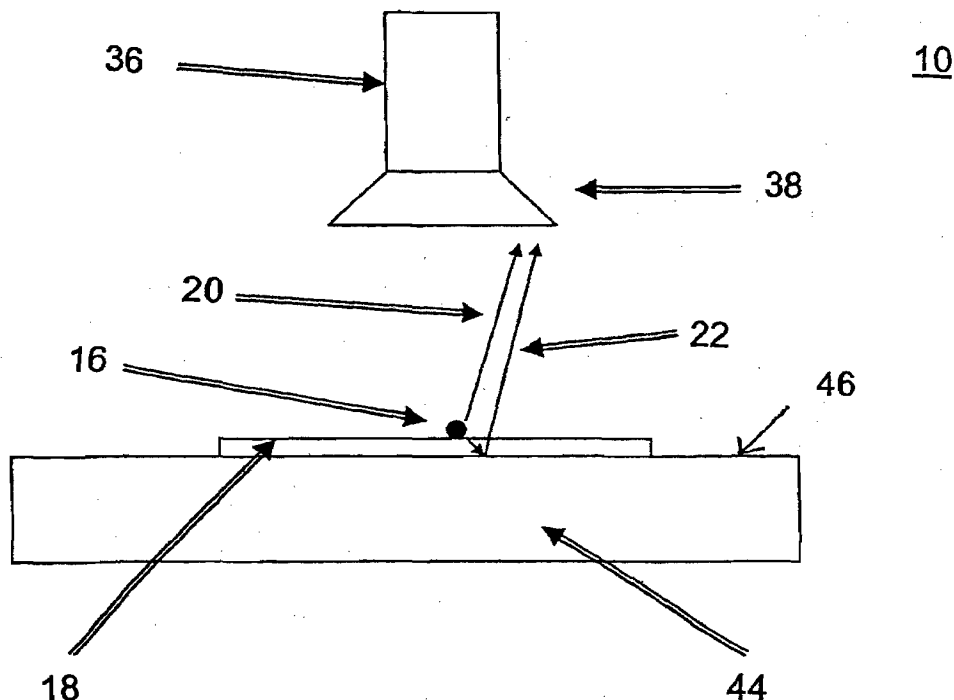
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[Continued on next page]

(54) Title: IMPROVED MICROARRAY READER



(57) Abstract: An optical imaging apparatus for imaging a sample carried on a transparent substrate is disclosed. The apparatus includes an energy source for generating detectable light from the sample and associated imaging and detection means. The amplitude of light emitted from the sample and detected by the imaging system is increased by the provision of a reflective surface below the imaged sample.



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IMPROVED MICROARRAY READER

FIELD OF THE INVENTION

5 The present invention relates to an optical imaging device, and in particular, to a device for use in reading a microarray of samples, such as an array of chemiluminescent or fluorescent sample regions on a substrate.

BACKGROUND OF THE INVENTION

10 There is a growing emphasis, in the fields of drug screening, nucleic acid sequencing and analysis, and protein engineering, on preparing and reading high density arrays of chemical or biological species. Such arrays can now be prepared efficiently by massive parallel schemes, such as the selective photomask techniques disclosed in U.S. Pat. No. 5,143,854. Microarrays of this type are typically formed in a planar area of
15 between about 4 to about 100 mm², and may have densities of up to several hundred thousand or more distinct array members/cm².

 In the usual application, the members of the microarrays are libraries of different polynucleotides, oligonucleotides, proteins, polypeptides or small-molecules with different R-group permutations, where each array sequence or permutation is position addressable,
20 i.e., each array position corresponds to a known sequence or permutation.

 In use, an array surface is reacted with one or more analytes, such as polynucleotide analytes, receptor proteins, or antiligand molecules, under conditions that promote specific, high-affinity binding of the analyte molecules to one of more of the array members. The goal is to identify one or more position-addressable members of the library
25 array which bind to the analyte, as a method of screening for array compounds which bind to the analyte or, in the case of oligonucleotide arrays, as a method of detecting array members which can hybridize with the analyte molecule(s).

 Typically, the analyte is labeled with a detectable reporter such as a chemiluminescent or fluorescent tag, which labels the one or more array regions where
30 analyte binding to the array occurs. In relatively sophisticated schemes, two or more analytes are labeled with distinct tags and the extent of analyte binding to the array members can be quantitated according to the level of chemiluminescence or fluorescence at array binding positions.

A variety of optical scanning devices have been proposed for reading microarrays of this type. U.S. Pat. No. 5,324,633, for example, describes a confocal fluorescence microscope device in which a laser light beam is focused by a lens system onto a small region of a substrate (beam spot less than the area of each array-member region). The same lens system is used to image fluorescence emission from the illuminated region onto a photodetector through a dichroic mirror. An X-Y movable stage functions to position each array region in the substrate successively in the illumination area of the laser beam.

Scanning confocal microscopes designed to correct chromatic aberration that occurs because of the different wavelengths of the illumination beam of fluorescence signal from the sample have also been proposed, e.g., U.S. Pat. Nos. 5,296,700 and 5,260,578. Even with relatively elaborate lens systems, however, scanning confocal microscopes have a number of inherent features that limit sample resolution and the signal-to-noise ratio achievable.

Charge-coupled devices (CCDs) are the key components in digital imaging cameras. High resolution cameras may be constructed around these highly sensitive devices with a suitable lens system and associated electronics. CCDs offer high quantum efficiency and sensitivity, high resolution, and a dynamic range exceeding 100 dB. However, even further increases in signal are desirable.

SUMMARY OF THE INVENTION

The current invention provides an optical imaging apparatus for imaging a sample on a transparent substrate, comprising an energy source effective to stimulate detectable light from the sample, imaging and detection means for detecting the light emitted from the stimulated sample, and a sample substrate holder, wherein a reflective surface is located below the sample to reflect emitted light into the detection means. The apparatus is preferably used in a microarray reader with an CCD detection system.

The use of the reflective surface has been found to increase signal by as much as 80%.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of one embodiment of a microarray reader in accordance with the invention;

FIG. 2 is a schematic representation of a second embodiment of a microarray reader in accordance with the invention.

DETAILED DESCRIPTION OF THE INVENTION

5 The current invention provides an optical imaging apparatus for imaging a sample on a transparent substrate, comprising an energy source effective to stimulate detectable light from the sample, imaging and detection means for detecting the light emitted from the stimulated sample, and a sample substrate holder, wherein a reflective surface is located below the sample to reflect emitted light into the detection means. The apparatus
10 is preferably used in a microarray reader with an CCD detection system.

 The reflective surface may be integrated into the sample substrate holder, detachably mounted on the sample holder, or be a component of the transparent substrate, e.g. a mirrored microscope slide. The reflective surface may be made of any material capable of reflecting light and useful in the relevant environment, however, the preferred
15 reflective surface is an aluminum, silver or mirrored glass surface.

 Preferably the apparatus of the invention is adapted for reading an array of samples and is provided with the requisite mechanical and electronic components for doing so. Reading may be linear, two dimensional or in any pattern selected by the operator.

 The energy source is one which will stimulate light emission from the sample, such
20 as a filtered light source, a laser or an infrared light beam. The samples may be on the upper surface of the substrate (Figure 1) or on the lower surface of the substrate (Figure 2). The latter represents a preferred embodiment since emitted and reflected light are coincident.

 The excited light may be produced by chemiluminescence or fluorescence.

25 The detection means may be a charge-coupled device, a charge injection device, a photodiode array or a scanner.

 Likewise, the invention provides a method for imaging a sample on a transparent substrate by the steps of a) irradiating said sample with an energy source effective to produce detectable light from said sample, b) providing a reflective surface below the
30 sample to reflect emitted light into the detection means, and c) detecting the light emitted from said sample in response to irradiation.

FIG. 1 is a schematic view of an optical scanning apparatus or microscope 10 constructed in accordance with the invention. The microscope includes a beam generator 12, including a light source and focusing lens, for generating and focusing a sample-illuminating beam 14 onto a sample substrate 18.

5 The light source in the apparatus is preferably a filtered light source or a laser for producing a coherent light beam at a selected wavelength, typically a UV or low-visible wavelength corresponding to the fluorescence excitation wavelength of a given fluorescent sample reporter. One exemplary light source is an argon laser. The light source and focusing lens are also referred to herein, collectively, as a beam generator.

10 A second or imaging lens system in the apparatus is designed to image light emitted from the sample 16, in response to illumination from beam 14, in an imaging plane indicated at 32. More specifically, this plane, which is normal to the plane of FIG. 1, is defined by a series of light emission axes, each representing the axis of light emitted from an illuminated region of the sample, and directed through the imaging lens system; directly emitted light 20 is detected along with reflected light 22.

15 With reference to FIGS. 1 and 2, light from the imaging system is focused onto a detector 36 for measuring emitted light from the sample surface. In the embodiment shown, the detector is a commercial photocell having a spatially non-resolving light-detecting surface 38, for detecting and spatially integrating total light impinging on the detecting surface. One suitable detector of this type is a conventional photomultiplier tube. Alternatively, the detector may include a one- or two-dimensional arrays of light detecting elements, such as provided in a standard charge coupled device.

20 With reference again to FIG. 1, the apparatus of the invention also includes a stage or sample substrate holder 44 having mirrored surface 46 on which substrate 18 is supported. The stage is movable in the direction along an axis corresponding to the plane of FIG. 1, normal to the direction of the illumination beam. The stage is movable in increments corresponding to adjacent linear arrays in a two-dimensional array pattern on a substrate, and typically in increments in the range of 1-20 microns. Microscope stages and actuators for incremental stage movement in a single direction are commercially available.

30 As one example, the substrate microarray may be a high density, two-dimensional array of different oligonucleotides suitable for use in sequencing by hybridization or detection of mutational forms of analyte nucleic acid. A solution of fluorescent-labeled

nucleic acid analyte is placed on the microarray under selected stringency conditions, leading to hybridization of the analyte with complementary-sequence oligonucleotides in the array, and fluorescent labeling in the array regions where such binding occurs. The substrate is then washed to remove unbound and non-specifically bound analyte.

5 As another example, the microarray may be prepared to include a high density array of different polypeptides which collectively make up a combinatorial peptide library. To this array is added a fluorescent-labeled receptor or anti-ligand analyte which may bind with high affinity to one or more of the library members. After exposing the array surface to the labeled target, the surface is washed to remove unbound and weakly bound target,
10 leaving fluorescent labeling at high-affinity regions of the microarray only.

Other types of one- or two-dimensional microarrays, such as small-molecule library arrays, arrays of single clonal cells, and the like are also suitable.

After labeling, the substrate is placed on the microscope stage for scanning and position mapping. In the configuration shown in FIG. 2 the sample substrate is a
15 transparent glass slide 50 having a microarray formed on its lower surface 52, and consisting of a two-dimensional array of regions. Mirrored surface 54 on substrate holder 56 reflects light beam 58 to the detector along with emitted light beam 60.

In a typical scanning operation, the stage is moved to position the scanning plane to correspond to one of the linear arrays. The illumination beam is now scanned across the
20 linear array, exciting fluorescence light emission in each region in the array where labeled analyte is bound. The emitted light is imaged onto the photodetector and the intensity of light emission is measured. The measured intensity associated with each mirror position, or alternatively, with each sensing element in a multi-element photodetector, is recorded and stored with the associated region in the scanning plane.

25 After the regions in a linear array are scanned, the stage is moved to place the scanning plane to correspond to the next linear array, and this array is now scanned and recorded as above.

While the invention has been described with respect to specific embodiments and applications, it will be appreciated that various changes and modifications may be made
30 without departing from the invention.

We claim:

1. An optical imaging apparatus for imaging a sample on a transparent substrate, comprising:
 - an energy source effective to stimulate detectable light from said sample,
 - 5 imaging and detection means for detecting the light emitted from said stimulated sample, and
 - a sample substrate holder,
 - wherein a reflective surface is located below the sample to reflect emitted light into the detection means.
- 10 2. The apparatus of claim 1, wherein said reflective surface is integrated into the sample substrate holder.
3. The apparatus of claim 1, wherein said reflective surface is detachably mounted on the sample holder.
4. The apparatus of claim 1, wherein said reflective surface comprises the lower
15 surface of the transparent substrate.
5. The apparatus of claim 1, wherein said reflective surface is an aluminum, silver or mirrored glass surface.
6. The apparatus of claim 1, further comprising means for scanning an array of samples.
- 20 7. The apparatus of claim 1 wherein said energy source is adapted to excite samples on the upper surface of the substrate and said imaging means is adapted to image light received from the upper surface of the substrate.
8. The apparatus of claim 6 wherein said energy source is adapted to excite samples on the lower surface of the substrate and said imaging means is adapted to image
25 light received from the lower surface of the substrate.
9. The apparatus of claim 1, wherein said energy source is effective to excite sample light emission at a selected wavelength, and said detector is effective to detect light emission at a different wavelength.
10. The apparatus of claim 9, wherein the excited light is produced by
30 chemiluminescence or fluorescence.
11. The apparatus of claim 1 wherein the detection means is a charge-coupled device, a charge injection device, a photodiode array, or a scanner.

12. A method for imaging a sample on a transparent substrate, comprising:
- a) irradiating said sample with an energy source effective to produce detectable light from said sample,
 - b) providing a reflective surface below the sample to reflect emitted light into the
5 detection means, and
 - c) detecting the light emitted from said sample in response to irradiation.
13. The method of claim 12 wherein the sample is positioned on the upper surface of the substrate.
14. The method of claim 12 wherein the sample is positioned on the lower surface
10 of the substrate, whereby emitted light passes through the substrate.
15. The method of claim 12 comprising imaging an array of samples.

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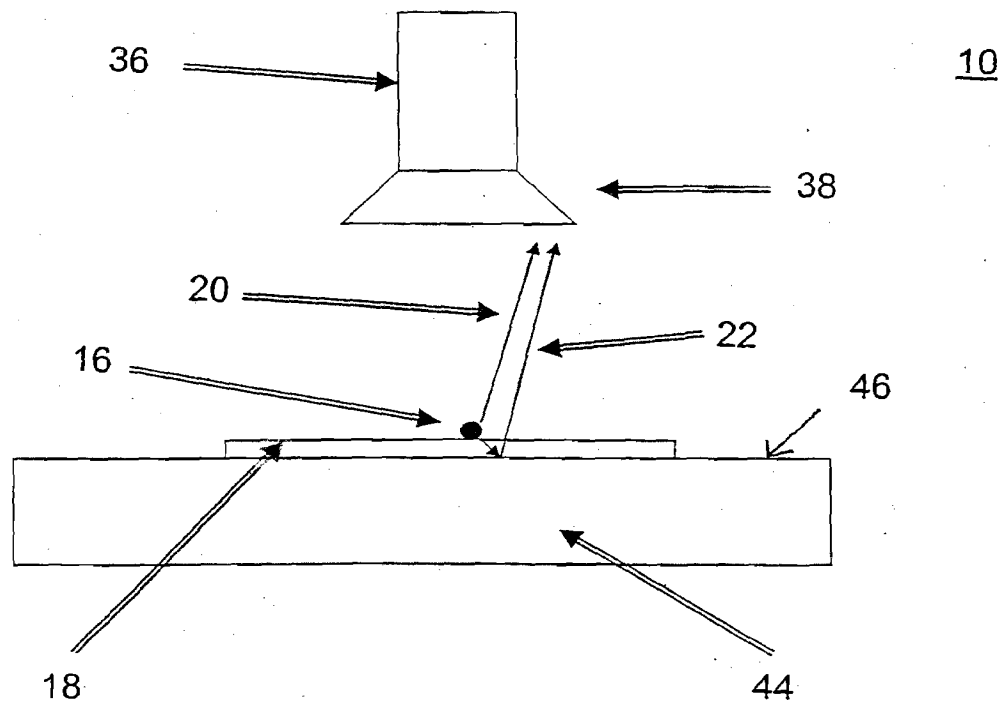


FIG. 1

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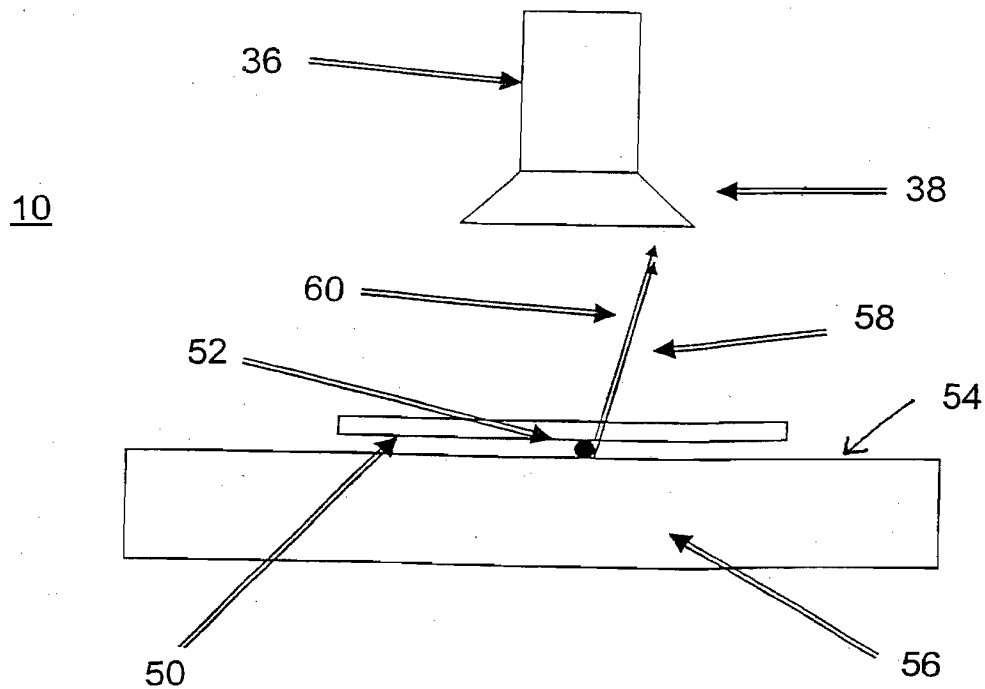


FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/03520

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G01N21/64 G02B21/34 G02B21/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 008 892 A (KAIN ROBERT C ET AL) 28 December 1999 (1999-12-28) column 1, line 5 - line 12 column 3, line 53 - line 66 column 6, line 44 - column 7, line 8; figures 1-4	1-7, 9-13, 15
X	EP 0 814 593 A (FUJI PHOTO FILM CO LTD) 29 December 1997 (1997-12-29) page 11, line 32 - line 55 page 12, line 43 - line 49; figure 1	1, 5-7, 9-13, 15
E	DE 100 24 247 A (FUJI PHOTO FILM CO LTD) 23 November 2000 (2000-11-23) column 4, line 8-30; figures 2-4	1, 5-15
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 01/03520

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 30 42 535 A (WANG WEI KUNG) 11 February 1982 (1982-02-11) page 12, line 15 - line 24; figures 1,2 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/03520

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6008892 A	28-12-1999	EP 1012578 A WO 9853304 A US 6177990 B	28-06-2000 26-11-1998 23-01-2001
EP 0814593 A	29-12-1997	JP 10003134 A US 5900640 A	06-01-1998 04-05-1999
DE 10024247 A	23-11-2000	JP 2000329769 A	30-11-2000
DE 3042535 A	11-02-1982	NONE	